(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 1 786 480 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
21.09.2016 Bulletin 2016/38

(21) Application number: **05756128.4**

(22) Date of filing: **07.07.2005**

(51) Int Cl.:
A61L 15/28 *(2006.01)* ***A61L 15/42*** *(2006.01)*

(86) International application number:
PCT/DK2005/000475

(87) International publication number:
WO 2006/005340 (19.01.2006 Gazette 2006/03)

(54) HAEMOSTATIC COMPOSITION COMPRISING HYALURONIC ACID

HÄMOSTATISCHE ZUSAMMENSETZUNG MIT HYALURONSÄURE

COMPOSITION HEMOSTATIQUE COMPRENANT DE L'ACIDE HYALURONIQUE

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IS IT LI LT LU LV MC NL PL PT RO SE SI SK TR

(30) Priority: **09.07.2004 DK 200401095**

(43) Date of publication of application:
23.05.2007 Bulletin 2007/21

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- **YOUNG SEON CHOI ET AL: "STUDIES ON GELATIN-CONTAINING ARTIFICIAL SKIN: II. PREPARATION AND CHARACTERIZATION OF CROSS-LINKED GELATIN-HYALURONATE SPONGE", JOURNAL OF BIOMEDICAL MATERIALS RESEARCH, WILEY, NEW YORK, NY, US, vol. 48, no. 5, 1 January 1999 (1999-01-01), pages 631-639, XP009007040, ISSN: 0021-9304, DOI: 10.1002/(SICI)1097-4636(1999)48:2<159::AID -JBM10>3.0.CO;2-1**

EP 1 786 480 B1

Description

TECHNICAL FIELD

[0001] The present invention relates to a haemostatic composition comprising gelatine, and hyaluronic acid or a derivative thereof. The haemostatic compositions described herein have superior haemostatic properties as compared to conventional compositions.

BACKGROUND

[0002] When blood vessels are injured by physical traumas including surgical interventions, bleedings will occur. Dependent on the extent of the injury, bleedings may result in losses of blood which can affect the normal function of the individual or, in cases of bleedings occurring in osseous non-expandable cavities, the accumulation of extravasated blood may cause damages of soft tissues due to increased pressure. If bleedings are left alone they will eventually be arrested by a normally occurring physiological process characterised by a chain of events involving the combined activity of vascular, platelet, and plasma factors. This process is referred to as a physiological haemostasis, an important element of which is blood coagulation which is described below. In the case of a minor superficial bleeding, this physiological haemostasis is adequate for the arrest.

[0003] Blood coagulation may be described as occurring in the following steps.

(1) The formation of an activator of prothrombin, which is a precursor of the plasma serine protease thrombin. The prothrombin activator is a complex of an enzyme factor Xa and two cofactors: factor Va and procoagulant phospholipids, both present on the surface of activated platelets. Furthermore, the presence of calcium ions is necessary for the function of the activator.

(2) The cleavage by the above activator system of prothrombin into two fragments, one of which is the enzyme thrombin.

(3) The conversion by thrombin of the plasma precursor fibrinogen to the clotting substance fibrin. This process involves several steps, the first of which comprises the cleavage of small peptides from fibrinogen, whereby fibrin monomers are formed, which then polymerise to form insoluble fibrin polymers. As a final step, thrombin activates the plasma factor XIII, an enzyme that catalyses the formation of covalent bonds between fibrin molecules, thereby cross-linking the molecules to form a firm clot resistant to dissolution.

[0004] In the above step (1) leading to the formation of the prothrombin activator system, several plasma proteases are involved in a cascade of proteolytic events. These blood coagulation factors are currently referred to by using Roman numerals, such as factor VII, factor VIII, factor IX, factor XI, and factor XII. The cascade involves sequential proteolytic activations of the next enzyme in the cascade. Thus activated blood coagulation factors are designated by their Roman numerals followed by an "a", such as factor VIIa, factor VIIIa, factor XIIIa or factor IXa.

[0005] However, bleedings emerging from more extensive injuries, especially such injuries which involve larger arteries or when seeping bleedings occur from larger mucosal surfaces or in cavities without drainage, require the adoption of surgical and/or medical haemostatic measures. Surgical arrest of bleeding comprises ligation or suture of disrupted blood vessels, plugging by using tampons in cavities, coagulating tissue surfaces including their exposed disrupted blood vessels by heated instruments or by the application of cauterising agents or heated air. Surgical haemostasis may also be aided by the application at the injured site of appropriately sized blocks, plates, or films of biologically absorbable haemostatic sponges. Powders or flakes, which are typically wetted before application to create a paste, have also been used.

[0006] In this context, the term "sponge" is understood to mean a porous structure characterised in that the structure is reticulate and has an inner surface considerably larger than its outer surface, that it contains hollow spaces within the reticulate structure, and that it can absorb many times its own weight in liquids.

[0007] Such haemostatic sponges or compositions are useful for enhancing the arrest of bleedings in several instances of surgical interventions or other injuries such as in surgery of large abdominal organs (liver, spleen, or intestines); in lung surgery; in neurosurgery to prevent pressure damages of the cerebral or nerve tissues; in orthopaedic surgery during which extensive haemorrhages frequently occur which are difficult to arrest by other means; in vascular surgery to arrest seeping bleedings from the sites of suturing; in oral or dental surgery such as extraction of teeth; and in nose-bleeding (epistaxis).

[0008] It is currently believed that the haemostatic effect (or mode of action) of a sponge is linked to sponge porosity and the sponge's ability to absorb blood. A conventional gelatine sponge adheres to the bleeding site and absorbs

approximately 45 times its own weight. Due to the uniform porosity of a conventional gelatine sponge, blood platelets are caught and the coagulation cascade is activated transforming soluble fibrinogen into a net of insoluble fibrin which stops the bleeding. Thus, a good capacity to absorb is believed essential for the mode of action of conventional gelatine sponges.

[0009] As mentioned above, a conventional gelatine sponge's ability to act as a haemostat is related to its ability to absorb, whereby the volume of the sponge inevitably will increase. However, swelling of the sponge can lead to adverse events if the sponge is not used according to the instructions. Normally, the instruction for use includes the phrase: "When placed into cavities or closed tissue spaces, minimal preliminary compression is advised and care should be exercised to avoid overpackaging. The gelatine sponge may swell to its original size on absorbing fluids creating the potential for nerve damage". Nevertheless, adverse events have taken place in the past, and the UK Medical Device Agency as well as the FDA have paid much attention to this drawback of conventional sponges.

[0010] Accordingly, there is a need for haemostatic sponges which, while maintaining a sufficient blood arresting (haemostatic) effect, swell to a much lesser extent than conventional haemostatic sponges. Evidently, such sponges would constitute a safer product.

[0011] The present disclosure deals with haemostatic compositions with improved properties and methods of producing said compositions.

[0012] The present inventors have surprisingly found that the haemostatic compositions as disclosed herein are more efficient in arresting bleeding than conventional compositions, such as Surgifoam®, Surgifoam® powder or Gelfoam® powder, i.e. the haemostatic properties of the compositions as disclosed herein are improved compared to conventional compositions.

[0013] Furthermore, the present inventors have solved the above-mentioned problem of swelling by incorporating hyaluronic acid (HA), or a derivative thereof, into a haemostatic composition.

[0014] Surprisingly, it has been found that swelling of a haemostatic sponge according to the present disclosure is considerably reduced compared to conventional sponges, such as Surgifoam®. Evidently, the haemostatic sponges described herein are safer to use than conventional sponges.

[0015] The above-mentioned properties, i.e. decreased tendency to swell and improved haemostatic properties, are attributed to the presence of HA, or derivatives thereof. HA, and derivatives thereof, are known to confer anti-adhesive properties to sponges as described in e.g. Laurent et al. Am J Otolaryngol; 7:181-186, 1986; US 6,548,081; US 6,099,952; US 5,503,848; US 5,700,476; EP 1 022 031 A1; WO 94/17840. Nevertheless, the surprising effects of conferring improved haemostatic properties to the composition and reducing the ability of a sponge to swell, have not been described in any of the above-identified prior art documents.

[0016] In summary, the haemostatic compositions and in particular a haemostatic sponge as disclosed herein contain numerous advantages as compared to conventional and commercially available haemostatic compositions and sponges:

i) reduced swelling, thereby rendering the sponge safer to use,

ii) improved haemostatic properties, and

iii) improved anti-adhesive properties, thereby reducing post-operative adhesion of tissues.

[0017] GB2393120 discloses a wound dressing composition, such as a sponge, comprising chitosan and an oxidized cellulose. The wound dressing is especially useful for the treatment of chronic wounds.

[0018] WO 90/13320 discloses a haemostatic sponge comprising gelatine and thrombin. The thrombin is added to the sponge by injection at a multiplicity of sites.

[0019] US 4,280,954 discloses a cross-linked collagen-mucopolysaccharide composite material useful in surgical sutures and prostheses, in particular where blood compatibility is required. The mucopolysaccharide is present in an amount sufficient to provide 6-15 wt % of said mucopolysaccharide in said collagen-mucopolysaccharide product.

[0020] Choi et al. (J. Biomed. Mater. Res. 48(5) 631-639 (1999)) discloses a gelatine-hyaluronate sponge with weight ratios of 9:1; 7:3, and 5:5 of gelatine to hyaluronate cross-linked with EDC.

SUMMARY OF THE INVENTION

[0021] In a first aspect, the present invention relates to a haemostatic composition comprising gelatine and hyaluronic acid or a derivative thereof, wherein said hyaluronic acid or derivative thereof, is incorporated into said composition to a final content of at least 10% (w/w) hyaluronic acid, wherein said composition does not comprise a chemical cross-linking agent or residues thereof, and wherein said composition is obtained by treating a mixture of gelatine, hyaluronic acid or a derivative thereof and a solvent with dry heat at a temperature of from 110 to 200°C.

[0022] In a second aspect, the present invention relates to a method of producing a haemostatic composition comprising

the steps of:

i) mixing gelatine, hyaluronic acid or a derivative thereof and a solvent
ii) treating the mixture obtained in step i) with dry heat at a temperature between 110-200°C.

[0023] Also disclosed herein is a composition obtainable by the above-mentioned method.
[0024] Also disclosed herein are methods for producing the haemostatic compositions of the present disclosure as well as their medical uses.

DETAILED DESCRIPTION

[0025] The present invention is as defined in the claims.
[0026] The general effect of haemostatic compositions is to enhance the physiological blood coagulation process thereby reducing the time elapsing from opening of the blood vessels until a firm blood clot has been formed. This period is generally referred to as "the time to haemostasis". In this context, the term "haemostatic" should be understood to mean the effect of an object or an agent, which reduces the time to haemostasis, thereby promoting haemostasis.
[0027] As indicated above, the present disclosure is, in its broadest aspect, directed to a haemostatic composition comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof.
[0028] In another embodiment the present disclosure relates to a haemostatic composition obtainable by a method of producing a haemostatic composition comprising the steps of:

i) mixing a biologically absorbable material and hyaluronic acid or a derivative thereof and a solvent
ii) treating the mixture obtained in step i) with dry heat at a temperature between 110-200°C.

[0029] The particulars of this method may be as described below.
[0030] In a particular embodiment of the present disclosure said composition may be in the form of a sponge, i.e. the haemostatic composition may be a sponge, wherein the term "sponge" is understood to mean a porous structure characterised in that the structure is reticulate and has an inner surface considerably larger than its outer surface, that it contains hollow spaces within the reticulate structure, and that it can absorb many times its own weight in liquids.
[0031] The biologically absorbable material may be any material which is known to be suitable for preparation of sponges, powders or flakes and, at the same time, being biologically absorbable. Examples of suitable biologically absorbable materials include gelatine, collagen, chitin, chitosan, alginate, cellulose, e.g. oxidised cellulose, oxidised regenerated cellulose, carboxymethylcellulose (CMC) or hydroxyethylcellulose (HEC), polyglycolic acid, polyacetic acid, derivatives thereof and mixtures thereof. It will be understood that various forms thereof, such as linear or cross-linked forms, salts, esters and the like may also be used as the biologically absorbable material to be included in the haemostatic sponges as disclosed herein. In one embodiment of the present disclosure the biologically absorbable material may be solid, wherein the term "solid" refers to the thermodynamic phase of the compound.
[0032] "Biologically absorbable" is a term which in the present context is used to describe that the materials of which the said haemostatic compositions are made can be degraded in the body to smaller molecules having a size which allows them to be transported into the blood stream. By said degradation and absorption the said sponge materials will gradually be removed from the site of application. For example, gelatine can be degraded by proteolytic tissue enzymes to absorbable smaller molecules, whereby the gelatine sponge when applied in tissues typically is absorbed within about 3-6 weeks and when applied on bleeding surfaces and mucous membranes typically within 3-5 days.
[0033] In a preferred embodiment of the present disclosure, the biologically absorbable material is gelatine. Gelatine is preferred since gelatine is highly biologically absorbable. Furthermore, gelatine is highly biocompatible, meaning that it is non-toxic to an animal, such as a human being, when/if entering the blood stream or being in long-term contact with human tissues.
[0034] The gelatine typically originates from a porcine source, but may originate from other animal sources, such as from bovine or fish sources. The gelatine may also be synthetically made, i.e. made by recombinant means. In an interesting embodiment of the present disclosure, the gelatine is prepared by denaturing collagen. Gelatine is typically prepared this way by denaturing collagen by chemical treatment with acids, bases, solvents, aldehydes, urea, or detergents such as sodium dodecyl sulphate and guanidine hydrochloride. By the above-mentioned denaturation procedure, the characteristics of the collagen molecule are modified, thereby resulting in an increase of water solubility. Furthermore, the gelatine may be stabilised, which is considered advantageous in relation to the use of the sponge as a haemostat, the mechanical strength of the structure being greatly increased as compared to a non-stabilised gelatine structure which when becoming moistened will become dissolved and thereby collapse. In contrast, a stabilised gelatine sponge will retain its structure for a considerable period of time after application to a bleeding site. Methods of stabilising such sponges include treatment with a chemical cross-linking agent or with dry heat as described in the subsequent paragraphs.

[0035] Even though gelatine, in particular prepared by denaturing collagen as defined above represents a particularly suitable embodiment of the present disclosure, it will be understood that other biodegradable materials currently used for haemostatic purposes, such as collagen, chitin, chitosan, alginate, cellulose, e.g. oxidised cellulose, regenerated oxidised cellulose, carboxymethylcellulose (CMC) or hydroxyethylcellulose (HEC), polyglycolic acid, polyacetic acid, derivatives thereof and mixtures thereof, said materials being in their native form or structurally modified, may also be used in certain embodiments of the present disclosure.

[0036] As indicated previously, the advantageous properties of the haemostatic sponges of the present disclosure are attributed to the presence of HA, or derivatives thereof, in the sponge. Another inherent advantage of HA, or a derivative thereof, is the excellent biologically absorbable and biocompatible properties of the molecule.

[0037] HA is a natural heteropolysaccharide consisting of alternate residues of D-glucuronic acid and N-acetyl-D-glucosamine. It is a linear polymer having a molecular weight ranging from about 50 to about 13,000 kDa, depending on the source it is obtained from and on the method of preparation. HA is present in nature in the preicellular gels, in the fundamental substance of connective tissue in vertebrate organisms (of which it is one of the main components), in the synovial fluid of joints, in the vitreous humor and in the umbilical cord. HA plays an important role in the biological organism as mechanical support for the cells of many tissues such as the skin, the tendons, the muscles and the cartilage. It is the main component of the extracellular matrix, but it has other functions such as hydration of tissues, lubrication as well as cell migration and differentiation. A suitable molecular weight for the purposes described herein will be in the range of from 50 to 5,000 kDa, such as in the range of from 50 to 4,000 kDa, e.g. in the range of from 100 to 3,000 kDa. In a particular preferred embodiment of the present disclosure, the HA, or a derivative thereof, has a molecular weight in the range of from 250 to 3,500 kDa, more preferably in the range of from 500 to 2,500 kDa, such as in the range of from 500 to 2,000 kDa.

[0038] Optionally, the HA molecule may be cross-linked, e.g. by chemical or physical means. In a preferred embodiment of the present disclosure the employed HA is pH neutral, i.e. an aqueous solution of the employed HA exhibits a pH value in the range of from 5 to 9, preferably in the range of from 6-8, in particular in the range of from 6.5 to 7.5, such as about 7. The HA used in the present dsiclosure may be extracted from any source, for example from rooster comb. Alternatively the HA may be obtained by fermentation.

[0039] Derivatives of HA include, for example, esters of HA, as well as the derivatives described in US 5,356,883; US 6,548,081; US 4,851,521; US 6,027,741; US 2003 181689; EP 1 095 064; EP 0 341 745; WO 02/18450 and WO 2004/035629. In addition, the term "derivative" is also intended to cover hyaluronate salt, including, but not limited to, sodium hyaluronate, potassium hyaluronate, magnesium hyaluronate and calcium hyaluronate.

[0040] Specific examples of HA derivatives include the following HA derivatives:

HA salified with organic and/or inorganic bases,

Hyaff®, i.e. HA esters with alcohols of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series, with an esterification degree that may vary depending on the type and length of the alcohol used,

Hyadd®, i.e. amides of HA with amines of the aliphatic, araliphatic, cycloaliphatic, aromatic, cyclic and heterocyclic series, with an amidation degree that may vary depending on the type and length of the amine used,

Hyoxx®, i.e. percarboxylated HA derivatives obtained by oxidation of the primary hydroxyl group of the N-acetyl-D-glucosamine unit,

deacetylates of HA, i.e. derived from deacetylation of the N-acetyl-D-glucosamine unit, and

O-sulphated HA derivatives.

[0041] The haemostatic composition of the present disclosure may in particular contain at least 0.5% (w/w) of HA or a derivative thereof calculated on the basis of the total weight of the "water-free" composition, or at least 1% (w/w) of HA or a derivative thereof, or at least 2% (w/w) of HA or a derivative thereof, or at least 3% (w/w) of HA or a derivative thereof, or at least 5% (w/w) of HA or a derivative thereof, or at least 7% (w/w) of HA or a derivative thereof, or at least 8% (w/w) of HA or a derivative thereof, or at least 10% (w/w) of HA or a derivative thereof. In an interesting embodiment of the present disclosure, the haemostatic composition comprises at least 15% (w/w) of HA or a derivative thereof, such as at least 20% (w/w) of HA or a derivative thereof, e.g. at least 25% (w/w) of HA or a derivative thereof, preferably at least 30% (w/w) of HA or a derivative thereof, such as at least 35% (w/w) of HA or a derivative thereof, e.g. at least 40% (w/w) of HA or a derivative thereof.

[0042] Analogously, the haemostatic sponge of the present disclosure typically comprises at the most 99.5% (w/w) of said biologically absorbable material, such as at the most 99% (w/w) of said biologically absorbable material, or at the

most 95% (w/w) of said biologically absorbable material, or at the most 90% (w/w) of said biologically absorbable material, or at the most 85% (w/w) of said biologically absorbable material, such as at the most 80% (w/w) of said biologically absorbable material, e.g. at the most 75% (w/w) of said biologically absorbable material, preferably at the most 70% (w/w) of said biologically absorbable material, such as at the most 65% (w/w) of said biologically absorbable material, e.g. at the most 60% (w/w) of said biologically absorbable material.

[0043] As will be understood by the skilled person, a significant amount of the biologically absorbable material must, however, be present in the sponge in order to provide the sponge with satisfactory mechanical and structural properties, i.e. the amount of HA, or a derivative thereof, should preferably not be too high. Accordingly, in a preferred embodiment of the present disclosure, the sponge comprises at the most 90% (w/w) of HA or a derivative thereof, such as at the most 80% of HA or a derivative thereof, e.g. at the most 70% (w/w) of HA or a derivative thereof, preferably at the most 65% (w/w) of HA or a derivative thereof, such as at the most 60% (w/w) of HA or a derivative thereof, e.g. at the most 55% (w/w) of HA or a derivative thereof, in particular at the most 50% (w/w) of HA or a derivative thereof.

[0044] It is well known that for some compounds water molecules may be bound to the compound even when the compound is regarded as being "dry" and solid. In the context of the present disclosure the term "water-free" means that there is no water present, i.e. that water is absent. Thus even such water molecules which may be bound to a compound, as described above are not included when the term "water-free" is used, e.g. the term "water-free compound" refers to a compound excluding any water molecules which may be bound. For the haemostatic sponge of the present disclosure some residual water, such as up to about 10% (w/w) may be present in the sponge. This residual water is, however, not calculated as part of the total weight of the sponge. This means that when the sponge is said to contain a specified weight percent of a certain component (such as the HA, a derivative thereof or the biologically absorbable material) this weight percent is calculated on the basis of the total water-free weight of the sponge, i.e. on the total weight of the sponge excluding any water which may be bound e.g. as described above.

[0045] In one embodiment of the present disclosure the haemostatic sponge is dry. In this context the term "dry" refers to a sponge which may comprise some residual water but where the content of water in the sponge is in equilibrium with the water in the air. Thus the content of water is less or equal to the amount of water which can be present when it is in equilibrium with the water in the air.

[0046] In the currently most preferred embodiment of the present disclosure, the HA, or a derivative thereof, is incorporated in the haemostatic sponge. By the term "incorporated" is to be understood that the HA molecules are located more or less uniformly in the reticulate sponge structure, preferably in such a way that no or only few "hot spots" of the HA molecules can be found in the sponge. The presence of "hot spots" does not have any effect on the functionality of the sponge. Thus, the term "incorporated", when used herein, may be used synonymously with expressions like "absorbed", "admixed" and the like.

[0047] In another embodiment of the present disclosure, the HA, or a derivative thereof, is applied to one or more of the surfaces of the sponge. In a preferred embodiment of the present disclosure, the HA, or derivative thereof, is applied to the surface intended for being in direct contact with the bleeding site, i.e. in a preferred embodiment of the present disclosure the HA, or a derivative thereof, is applied to only one, two or three of the surfaces of the sponge. It will be understood that when the HA, or a derivative thereof, is applied to one or more of the surfaces of the sponges, the major part of the HA, or a derivative thereof, will be located on said surface(s). Nevertheless, a certain amount of HA, or a derivative thereof, may end up being incorporated into the sponge as the pore size of the sponge may be so that when a layer of HA, or a derivative thereof, is applied to the surface(s) of the sponge, the molecules of the HA, or a derivative thereof, may partially penetrate into the pores of the sponge.

[0048] Even though HA, or derivatives thereof, represents a particularly suitable embodiment of the present disclosure, also disclosed herein are polysaccharides with properties similar to HA, which may be used in certain embodiments of the present disclosure. Examples of polysaccharides which may substitute HA, or derivatives thereof, in the sponges of the present dislcosure, include the mucopolysaccharides, such as chitin, chitosan, chondroitin sulphate, dermatan sulphate, keratan sulphate as well as alginate.

[0049] As explained above, one particular advantage of the haemostatic sponge of the present disclosure is the anti-adhesive properties of HA, or a derivative thereof, which in turn has the advantage that post-operative adhesion of tissues may be avoided as explained in, e.g. US 5,548,081 and in Laurent et al. Am J Otolaryngol; 7:181-186, 1986.

[0050] The main advantage of the haemostatic sponges of the present disclosure is, however, their ability to provide an efficient haemostatic action while, at the same time, possessing a reduced tendency to swell. Swelling of haemostatic sponges may be measured by various methods. For example, the swelling tendency may be expressed as the sponge's capability to absorb water; the more water absorbed, the more swelling (the greater volume) is obtained.

[0051] As will be apparent from the experiments provided herein, the haemostatic sponge of the present disclosure has a reduced tendency to swell, i.e. to absorb water, when determined in accordance with USP 24. Accordingly, a preferred haemostatic sponge of the present disclosure absorbs less water, i.e. it swells to a lesser extent than an absorbable gelatine sponge, such as Surgifoam®. More particularly, in a preferred embodiment of the present disclosure, the ratio between the water absorbed by the haemostatic sponge of the present disclosure and the water absorbed by

an absorbable gelatine sponge, such as Surgifoam®, is at the most 0.95 when determined in accordance with USP 24. More preferably, the ratio is at the most 0.90, such as at the most 0.85, or at the most 0.80, or at the most 0.75, such as at the most 0.70, e.g. at the most 0.65, even more preferably at the most 0.60, such as at the most 0.55, in particular at the most 0.50.

[0052] Alternatively, the swelling properties may be assessed by soaking the haemostatic sponge in an excessive amount of distilled water. The sponge is subsequently picked up and air-dried for 10 minutes in order to drain out excess free water. The weight of the sponge before and after water absorption is used for calculating the swelling ratio: Swelling ratio = $(m_f - m_i)/m_i$, where m_f is the weight of the sponge after soaking and removing of excess water, and m_i is the initial weight of sponge before soaking.

[0053] Alternatively or in addition to the above tests, one may measure the expanding volume of a sponge in a graduated cylinder with distilled water; A known weight and volume of a sponge is pre-wetted and kneaded and quantitatively poured into the cylinder. The expanded volume is then measured and divided by the initial mass of the sponge, i.e. the weight of the sponge before pre-wetting.

[0054] In a preferred embodiment of the present disclosure, the sponge further comprises at least one blood coagulation factor, such as a blood coagulation factor selected from the group consisting of thrombin or a precursor thereof, factor Va, factor VIIa, factor VIIIa, factor IXa, factor Xa, factor XIa, factor XIIa, factor XIIIa and calcium ions. In a highly preferred embodiment of the present disclosure, said blood coagulation factor is thrombin or a precursor thereof, in particular thrombin itself. As seen from the examples the presence of thrombin in a sponge of the present disclosure decreases the time to haemostasis further.

[0055] The thrombin may be plasma-derived, typically from a mammal source, such as a human. Alternatively, the thrombin may be produced by recombinant means.

[0056] Thrombin as well as precursors thereof may be incorporated into a haemostatic sponge by conventional means known to the person skilled in the art and as carefully explained in WO 90/13320.

[0057] In case thrombin or a precursor thereof is incorporated into the sponge of the present disclosure, the sponge preferably further comprises a thrombin-stabilising agent, such as a thrombin-stabilising agent selected from the group consisting of naturally occurring amino acids, mono- or disaccharides, polyglycols, proteins and mixtures thereof.

[0058] By naturally occurring amino acids are understood any amino acid which is found in biologically produced proteins, including essential and non-essential dietary amino acids in their two stereoisomeric forms, such as arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, alanine, aspartate, cysteine, glutamate, glycine, proline, serine, tyrosine, glutamine, and asparagine. Preferred amino acids are selected from the group consisting of glycine, lysine and arginine.

[0059] Suitable monosaccharides may be selected from D- or L-forms of pentoses, such as ribose, arabinose, xylose, and lyxose and hexoses such as allose, altrose, glucose, mannose, gulose, idose, galactose, talose and derivatives thereof, e.g. pentosamines, hexosamines and glucoronic acid. Disaccharides may be selected from lactose, saccharose, maltose, fructose, and cellubiose, including derivatives thereof.

[0060] In a preferred embodiment of the sponge of the present disclosure, a polyvalent alcohol is used as a thrombin-stabilising agent. A suitable polyvalent alcohol may be selected from ethylene glycol, diethylene glycol, propylene glycol, glycerol, mannitol, inositol, xylitol, erythritol, pentaerythritol, pentitols, hexitols, such as sorbitol, and heptitols. Furthermore, polyglycols, such as polypropylene glycol and polyethylene glycols may be useful as thrombin-stabilising agents. Among the latter group of compounds polyethylene glycols having a molecular weight in the range of 400 - 20,000, such as about 6,000 are preferred.

[0061] In an interesting embodiment of the present disclosure, the HA, or derivative thereof, exerts in itself a thrombin-stabilising effect, in which case the haemostatic sponge does not contain any further thrombin-stabilising agents.

[0062] The mammalian body has an innate fibrinolytic system which is activated by deposition of fibrin. By dissolving fibrin, this system helps keep open the lumen of an injured blood vessel. However, in a situation where rapid haemostasis is aimed at, the fibrinolytic activity may counteract the haemostatic effect of a haemostatic adjunct, such as a haemostatic sponge according to the present disclosure. The fibrinolytic system involves the activation of plasminogen, a plasma precursor for an active proteolytic enzyme, plasmin, which is bound to lysine residues on the fibrin. Accordingly, it may be advantageous to have agents incorporated in the haemostatic sponge as disclosed herein, which have an anti-fibrinolytic effect. Specific examples include an anti-fibrinolytic agent selected from the group consisting of aprotinin, pepstatin, leupeptin, antipain, chymostatin, gabexate mesilate, fibronectin, ε-amino caproic acid and tranexamic acid. Most preferably, the anti-fibrinolytic agent is ε-amino caproic acid or tranexamic acid, in particular tranexamic acid.

[0063] In addition, the haemostatic sponge of the present disclosure may contain a buffering agent. Examples of buffering agents include alkaline metal salts, such as acetates, citrates, phosphates, hydrogen phosphates, carbonates, hydrogen carbonates, and succinates. Other useful buffering agents include imidazole, TRIS, and zwitteranionic buffering systems. Evidently, mixtures of the above-mentioned buffering agents may also be used.

[0064] Due to the superior swelling properties of the sponges describe herein, it will be appreciated that the sponge in addition to, or as an alternative to, providing a haemostatic effect, also may be used for local delivery of desirable

agents, thereby using the sponge as a delivery vehicle or matrix. The desirable agent may be incorporated in the sponge or applied to one or more of the surfaces of the sponge in a conventional way, e.g. by soaking, dipping, spraying the sponge of the present disclosure in or with a solution of the desirable agent or by other methods known to the skilled person. This can, e.g., be done by the clinician prior to use of the product by the clinician, or by the manufacturer.

[0065] Accordingly, in another aspect the present disclosure relates to a sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said sponge further comprises at least one desirable agent, in particular a desirable agent selected from the group consisting of surfactants, antimicrobial agents, antibacterial agents such as antiseptics and antibiotics, pain relieving agents, chemotherapeutics, anaesthetics, healing-promoting agents, vitamins, minerals, amino acids, proteins, growth factors, cells, enzymes, contrast agents, preservatives, emulsifiers, cross-linking agents to promote healing, etc.

[0066] In yet another aspect, the present disclosure relates to the use of a sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said sponge further comprises at least one desirable agent, for local delivery of said desirable agent.

[0067] In still another aspect, the present disclosure relates to a method for local delivery of a desirable agent to a patient in need thereof, said method comprising placing a sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said sponge further comprises at least one desirable agent, at the local site of the patient where said desirable agent is intended to be delivered. Thus the sponge comprising a biologically absorbable material and HA or a derivative thereof may in particular be used as a vehicle for delivery of an agent.

[0068] The term "local site" is intended to mean a part of a patient's body, in particular internal organs, such as a kidney, spleen, heart, etc.

[0069] In particular said desirable agent may be an antimicrobial agent. The antimicrobial agent may be selected from bactericidal or bacteriostatic agents, such as antibiotics and sulphonamides, antiviral compounds, antimycotic agents and anti-infectives. Antibiotics may be selected from e.g. β-lactams, penicillins, cephalosporins, monobactams, macrolides, polymyxins, tetracyclines, chloramphenicol, thrimethoprim, aminoglycosides, clindamycin, and metronidazole; sulphonamides may for example be selected from sulphadimidine or sulphadimethoxin; antimycotic agents may be selected from amphotericin B, ketoconazol and miconazol; and antiviral agent from idoxuridine andazidothymidin. Suitable antiinfectives may for example be selected from halogens, chlorohexidine, quarternary ammonium compounds and triclosan. Other examples of bactericidal or bacteriostatic compounds include silver ions, in particular in the form of silver ion complexes. Another example of a suitable antimicrobial agent is tobramycin which in particular may be used as a salt thereof such as tobramycin sulphate.

[0070] The desirable agent may also be a chemotherapeutic such as Carboplatin.

[0071] Surfactants may be selected from the group consisting of anionic surfactants, cationic surfactants, non-ionic surfactants and surface active biological modifiers.

[0072] Examples of anionic surfactants include surfactants selected from the group consisting of potassium laurate, triethanolamine stearate, sodium lauryl sulfate, sodium dodecylsulfate, alkyl polyoxyethylene sulfates, sodium alginate, dioctyl sodium sulfosuccinate, phosphatidyl glycerol, phosphatidyl inositol, phosphatidylserine, phosphatidic acid and their salts, glyceryl esters, sodium carboxymethylcellulose, bile acids and their salts, cholic acid, deoxycholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, and calcium carboxymethylcellulose. In particular sodium lauryl sulfate is preferred.

[0073] Examples of cationic surfactants include surfactants selected from the group consisting of quaternary ammonium compounds, benzalkonium chloride, cetyltrimethylammonium bromide, chitosans and lauryldimethylbenzylammonium chloride.

[0074] Examples of non-ionic surfactants include surfactants selected from the group consisting of polyoxyethylene fatty alcohol ethers, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene fatty acid esters, sorbitan esters, glycerol monostearate, polyethylene glycols, polypropylene glycols, cetyl alcohol, cetostearyl alcohol, stearyl alcohol, aryl alkyl polyether alcohols, polyoxyethylene-polyoxypropylene copolymers, polaxamines, methylcellulose, hydroxycellulose, hydroxy propylcellulose, hydroxy propylmethylcellulose, noncrystalline cellulose, polysaccharides, starch, starch derivatives, hydroxyethylstarch, polyvinyl alcohol, and polyvinylpyrrolidone.

[0075] Examples of biological surfactants include, e.g., albumin and casein.

[0076] Examples of preservatives include benzoic acid, sorbic acid, parabens (e.g. methyl-p-hydroxy benzoic acid, ethyl-p-hydroxy benzoic acid, propyl-p-hydroxy benzoic acid, butyl-p-hydroxy benzoic acid and mixtures thereof), benzyl alcohol, chlorhexidine or benzalkonium chloride.

[0077] In an interesting embodiment of the present disclosure, the haemostatic sponge of the present disclosure is equipped with a top sheet, i.e. at least one of the surfaces of said haemostatic sponge may be covered by a top sheet. In one embodiment of the present disclosure the top-sheet is not biodegradable. In one embodiment of the present disclosure, the top sheet is removable and constructed from a thin plastic film of e.g. polyethylene, polypropylene or other materials which are substantially water-impervious. The skilled person will be aware of other suitable materials having the desired and required mechanical properties for this purpose. As will be understood, such top sheets are

typically not biodegradable and should subsequently be removed. The materials mentioned above are typically transparent which, in turn, may give rise to problems in identifying the top sheet under and/or after surgery, in particular if the surgical area is covered or filled with a substantial amount of blood. Evidently, this increases the risk that the surgeon, or the staff assisting him, overlooks the presence of the top sheet. As will be understood, in case such a non-biodegradable top sheet is left in the body this may give rise to a severe clinical condition for the patient in question. Accordingly, in a preferred embodiment of the present disclosure, a dye is incorporated in or on the top sheet, or part of the top sheet, thereby improving the visibility of the top sheet.

[0078] In another, and even more preferred, embodiment of the present disclosure, the top sheet is prepared from a biodegradable material. Examples of suitable biodegradable materials include, for example, the polymeric materials mentioned in WO 2004/028583, page 6, line 3 to page 7, line 32. It will be understood that in this case the top sheet needs not necessarily be removed after surgery but may be left in the body.

[0079] The sponges of the present disclosure may be prepared in any desirable shape and/or dimension depending on the intended use and the available process equipment. Typically, however, the thickness of the sponge of the present disclosure will be in the range of 1-20 mm, such as in the range of 2-10 mm. Preferably, the thickness of the sponge is not less than 1 mm.

[0080] The haemostatic sponge is preferably subjected to a sterilisation treatment. Preferred methods of sterilisation include exposing the sponge to dry heat, ethylene oxide (EtO) or radiation, however other methods of sterilisation may be foreseen. Sterilisation with dry heat may typically be carried out by heating the sponges at a temperature between 110-200°C. In particular the temperature may be in the range of 110-160°C, e.g. in the range of 110-140°C, or in the range of 120-180°C, or in the range of 130-170°C, or in the range of 130-160°C, or in the range of 120-150°C. Furthermore, the period of time may in particular before a period between 15 minutes to 6 hours, such as between 1-4 hours, e.g. between 1,5-3 hours, or between 2-4 hours.

Methods for sterilisation with EtO are known to a person skilled in the art.

In yet another embodiment of the present disclosure the sterilisation may be carried out by application of radiation, such as beta-or gamma-radiation. The dose typically lies in the range of 10-60 kGy, e.g. 20-60 kGy or 25-50 kGy, or 15-25 kGy, or 15-20 kGy, in particular around 15 kGy, 20 kGy or 25 kGy. Such treatment will reduce the bioburden of the sponge, and may also add to cross-linking of the molecular chains in the product.

In a particular embodiment of the present disclosure the haemostatic sponge of the present disclosure has not been treated with a chemical cross-linking agent to stabilise the sponge but instead it has been treated with dry heat as described below. Thus in a preferred embodiment of the present disclosure the haemostatic sponge of the present disclosure does not comprise a chemical cross-linking agent. The term chemical cross-linking agent is to be understood as any compound which is capable of stabilising a sponge of a biologically absorbable material. Such compounds are sometimes referred to as "activating agents". Different compounds have been used to chemically cross-link such sponges and include but are not limited to aldehydes, in particular glutaraldehyde and formaldehyde, acyl azide, caboiimides, hexamethylene diisocyanate, polyether oxide, 1,4-butanedioldiglycidyl ether, tannic acid, aldose sugars, e.g. D-fructose, genipin and dye-mediated photo-oxidation. Specific compounds include but are not limited to 1-(3-dimethylaminopropyl)-3-ethylcarboiimide hydrochloride (EDC), dithiobis(propanoic dihydrazide) (DTP), 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDAC), Thus in a preferred embodiment of the present disclosure the haemostatic composition has not been treated with one of the above-mentioned chemical cross-linking agents, i.e. the haemostatic composition does not comprise any of the above-mentioned chemical cross-linking agents or residues thereof present as a result of having been used to stabilise the composition.

[0081] As indicated above, the haemostatic sponge of the present disclosure may be used as a medication. In particular, the haemostatic sponge of the present disclosure may be used for a haemostatic adjunct, or in the preparation for a haemostatic adjunct, in medical, veterinary or dental surgery. Accordingly, in a further aspect the present disclosure relates to a method of promoting haemostasis in a patient in need thereof, said method comprising applying a haemostatic sponge of the present disclosure onto at least a portion of the area where bleeding occurs. In a still further aspect the present disclosure relates to a method for arresting bleeding comprising applying to the site of bleeding a haemostatic sponge according to the present disclosure.

[0082] Before applying the haemostatic sponge to the surface, it is typically wetted in a saline solution. The present inventors have found that sponges which have been wetted before application have a lower time to haemostasis than when the same sponges are applied in dry form, i.e. without wetting the sponges before application. Thus in a preferred embodiment of the present disclosure said sponge is wetted before it is applied to the surface. The haemostatic sponge may then subsequently be applied directly to surfaces and optionally, after being applied to the surface, held in place by pressure, e.g. by means of pads, dressings, webs, films, etc. or by other materials normally used in the medical practice. A preferred material for holding the sponge in place after being applied to the surface is surgical gauze or cotton gauze, optionally wetted in saline.

[0083] The haemostatic sponge of the present disclosure may be used in an array of surgical procedures wherein bleeding control is desired, such as in orthopaedic procedures, e.g. in connection with laminectomy, total hip replacement

and hip revisions, knee surgery, spinal fusion, etc.; in cardiothoracic/cardiovascular procedures, such as in connection with CABGs, valve replacements, aotic surgery, abdominal aortic aneurisms, carotid endarterectomy and femoral-popliteal bypass, amongst others.

[0084] In another embodiment the haemostatic composition of the present disclosure is in the form of a powder or flakes. Such a composition is intended to include a composition comprising a powder or flakes of a biologically absorbable material and a powder or flakes of hyaluronic acid or a derivative thereof, i.e. a composition wherein the individual powder granules or flakes either comprises a biologically absorbable material or hyaluronic acid or a derivative thereof. Such compositions are furthermore intended to include compositions wherein the individual powder granules or flakes comprise both a biologically absorbable material and hyaluronic acid or a derivative thereof.

Thus in a further aspect of the present disclosure the original shape of the haemostatic sponges of the present disclosure may be modified. For example, the sponge of the present disclosure may be milled to a powder or flakes by methods known in the art, e.g. by means of rotary bed, extrusion, granulation and treatment in an intensive mixer, milling (e.g. by using a hammer mill or a centrifugal mill), or spray drying. Such powders or flakes may be used "as is" or may be pre-wetted with a liquid, such as saline, before use, thereby creating a paste.

[0085] The term "paste" may be used interchangeable with words like "gel", "suspension" and the like. In the present context, the term "paste" refers to a solid or semi-solid disperse system wherein the biologically absorbable material is dispersed in a liquid medium. The biologically absorbable material may also be referred to as a gel- or paste-forming agent. Furthermore, a paste is characterised by having a dynamic viscosity above that of water.

[0086] The paste may be obtained by suspending the particles of the biologically absorbable material (described above) in a liquid medium, in particular in an aqueous medium. Typically, about 1-20 ml liquid medium is employed per gram biologically absorbable material. The liquid medium is preferably an aqueous medium. More preferably the aqueous medium contains salts, such as sodium chloride, dissolved therein. Most preferably, the aqueous medium is saline.

[0087] Accordingly, in a further aspect the present disclosure relates to a powder composition comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof.

[0088] A still further aspect of the present disclosure relates to a paste comprising water, a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof. It will be understood that the weight percentages given is calculated on the basis of only solid material in the paste.

[0089] Details and particulars concerning the powder and paste aspects of the present disclosure will be the same as for the sponge aspect discussed above, and it means that whenever appropriate, the statements concerning amounts of HA, or a derivative thereof, present in the sponge, additional components present in the sponge, etc., will apply mutatis mutandis to the powder and paste aspects of the present disclosure.

[0090] In further aspects the present dsiclosure relates to methods for preparing the haemostatic compositions of the present disclosure.

[0091] In a particular embodiment the present disclosure relates to a method of producing a haemostatic composition comprising the steps of:

i) mixing a biologically absorbable material, hyaluronic acid or a derivative thereof and solvent
ii) treating the mixture obtained in step i) with dry heat at a temperature between 110-200°C.

[0092] Hyaluronic acid is generally considered to be unstable at high temperatures. It was therefore a surprise when the present inventors found that a sponge comprising hyaluronic acid prepared according to the above-mentioned method had a higher reduction in the bleeding intensity than a similar sponge without hyaluronic acid (as shown in example 6). Thus indicating that the HA was active even though it had been treated with dry heat. In the context of the present disclosure the term "dry heat" refers to the fact that the treatment is conducted without the presence of a saturated water vapour. Typically treatments at these temperatures are distinguished as being in the presence of a saturated water vapour, e.g. autoclaving, or without the presence of a saturated water vapour, i.e. with dry heat. The relevant period of time to treat the mixture with dry heat in step ii) depends on the temperature but may typically be in the range of 15 minutes to 6 hours, such as in particular between 30 minutes and 4 hours, e.g. between 1-4 hours, or between 1-3 hours or between 1-2 hours.

[0093] The step of treating the mixture with dry heat has a number of functions including:

a) Stabilisation of the three-dimensional structure of the mixture
b) Sterilisation
c) Removal of endotoxins

[0094] The function of stabilisation is in particular important for haemostatic sponges because it makes the sponges less prone to disintegration and thereby affects the ability to handle the sponges.

[0095] The function of sterilisation and removal endotoxins is of course important for all forms of haemostatic compo-

sitions.

[0096] Said method may further comprise a further step of drying the mixture obtained in step i) before treating it according to step ii)

Details and particulars concerning how to mix the biologically absorbable material and hyaluronic acid or a derivative thereof and a solvent and how to dry the haemostatic composition may be performed as described below for the other methods of the present disclosure.

[0097] The present disclosure also relates to a haemostatic composition obtainable by the above-mentioned method. Previously chemical agents have been used to cross-link haemostatic sponges. An advantage of a haemostatic composition obtainable by the above-mentioned method is that it does not involve the use of a chemical cross-linking agent; hence residues of chemical agents in the composition are avoided.

It is to be understood that the details and particulars concerning the aspects of the haemostatic composition obtainable by the above-mentioned will be the same as for the sponge and powder and flakes aspects discussed above, and this means that whenever appropriate, the statements concerning the biologically absorbable material, amounts of HA, or a derivative thereof, present in the sponge, additional components present in the sponge, the form of the composition, etc., will apply mutatis mutandis to the aspects of the haemostatic composition obtainable according to the above-mentioned method.

[0098] The haemostatic composition comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof may be prepared by a number of different methods, which may depend on the form of the haemostatic composition, e.g. whether it is in the form of a sponge or a powder or flakes. Other aspects such as whether the hyaluronic acid is to be applied to a sponge or whether it is an integrated part of the sponge may also affect the choice of production method.

[0099] Hence if the haemostatic composition comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof is in the form of a sponge, said sponge may be prepared by one of the following methods although other methods of production are foreseen.

Thus in one embodiment the present disclosure also relates to a method for preparing a haemostatic sponge comprising a biologically absorbable material and hyaluronic acid or a derivative thereof, said method comprising the steps of:

i) Treating a sponge of a biologically absorbable material with dry heat at a temperature between 110-200°C
ii) soaking the sponge obtained in step i) in hyaluronic acid or a derivative thereof

[0100] Step i) treating the sponge with dry heat may be carried out as described above.

[0101] Without being bound by any theory the present inventors believe that by soaking the sponge into a solution of HA or a derivative thereof said HA or derivative thereof becomes an integral component of the sponge in contrast to method in which a solution of HA or a derivative thereof is applied to one or more surfaces of said sponge. However, this may of course depend on how long the sponge is soaked in the solution. Hence if the sponge is only soaked very briefly in the HA or a derivative thereof solution it may be similar to applying it to one or more surfaces of the sponge.

In a preferred embodiment of the present disclosure said method may comprise a further step of drying the sponge obtained in step ii). Said drying may be performed as described below.

[0102] In another embodiment, the present disclosure relates to a method for preparing a haemostatic sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said HA, or a derivative thereof, is incorporated in said sponge, said method comprising the steps of:

i) mixing a biologically absorbable material, hyaluronic acid or a derivative thereof and solvent;

ii) drying said mixture.

[0103] Said method may further comprise a step of stabilising the mixture obtained in step ii). Such methods of stabilisation include any of those methods described below, e.g. stabilisation with dry heat or with a chemical cross-linking agent.

[0104] In a particular embodiment of the present disclosure of any of the above-mentioned methods the mixing of the biologically absorbable material, hyaluronic acid or a derivative thereof and solvent may be performed by any of the following alternatives:

a) mixing a biologically absorbable material with hyaluronic acid or a derivative thereof and then subsequently adding a solvent
b) mixing a solution of a biologically absorbable material with a solution of hyaluronic acid or a derivative thereof
c) mixing a biologically absorbable material with a solution of hyaluronic acid or a derivative thereof
d) mixing a solution of a biologically absorbable material with hyaluronic acid or a derivative thereof.

[0105] Furthermore, said mixing may in particular be performed under mechanical influence, such as whipping, stirring, spinning, static mixing, motionless mixing or centrifugation.

[0106] A number of different static mixing systems exists, e.g. from Bollin Dale which design and manufacture a range of static mixing systems for blending of a single product. Static mixing is sometimes also called motionless mixing. By the application of a dosing system and inline injectors, colours, flavouring or acids can be introduced into the product flow.

[0107] Thus the method for preparing a haemostatic sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said HA, or a derivative thereof, is incorporated in said sponge, may in particular comprise the following steps:

i) providing a whipped or foamed solution of a biologically absorbable material, which may in particular be selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, e.g. oxidised cellulose, oxidised regenerated cellulose, carboxymethylcellulose (CMC) or hydroxyethylcellulose (HEC), polyglycolic acid, polyacetic acid, derivatives thereof and mixtures thereof;
ii) providing a solution of HA or a derivative thereof;
iii) mixing the solutions provided in i) and ii) above; and
iv) drying said mixture.

[0108] Said method for preparing a haemostatic sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said HA, or a derivative thereof, is incorporated in said sponge, may in particular further comprise a step of stabilising the sponge.

[0109] In a preferred embodiment of the present disclosure the above-mentioned solvent and/or solution is an aqueous solution. Said aqueous solution may in particular further comprise one or more salts, buffers or other suitable compounds, such as any of those described above.

[0110] The preferred biologically absorbable material is gelatine. It is in some cases necessary to foam, whip or subject the solution to other mechanical forces, so that the desirable texture is obtained. In a preferred embodiment of the present disclosure, the texture of the whipped or foamed solution resembles that of whipped cream. A suitable concentration of the biologically absorbable material will depend on the material of choice, but will typically be in the range of from 5 to 30% (w/v), such as in the range of from 10 to 20% (w/v). The temperature is preferably kept in the range of from 25°C to 60°C, more preferably in the range of from 35°C to 55°C.

[0111] In a preferred embodiment of the present disclosure said mixing may be performed according to embodiment b), i.e. by mixing a solution of a biologically absorbable material with a solution of hyaluronic acid or a derivative thereof. The solution of hyaluronic acid or a derivative thereof mentioned above may also preferably be provided in the form of a gel. The exact concentration of HA, or a derivative thereof, may largely depend on the HA or derivative used. Generally speaking, the solution should neither be too liquid, nor too viscous. The present inventors have found that good results are obtained using a concentration of from 1 to 5% (w/v), in particular of from 2 to 4% (w/v). In order to avoid cooling of the whipped or foamed solution of biologically absorbable material upon mixing, thereby potentially leading to an inhomogeneous mixture, the HA solution is preferably kept at an slightly elevated temperature, such as a temperature in the range of from 25°C to 50°C, e.g. in the range of from 25°C to 40°C, or in the range of from 25-35°C.

[0112] The mixing of the biologically absorbable material and a solvent is typically performed under mechanical influence in order to avoid clogging of the biologically absorbable material. Thus, during and/or immediately after mixing, the resulting mixture should preferably be whirled at high velocity, whipped, spinned, centrifuged or subjected to other kind of mechanical influence as will be known to the person skilled in the art. The solution of HA or a derivative thereof is subsequently mixed with said solution of the biologically material or a derivative thereof.

[0113] After mixing the resulting mixture may be poured into suitable trays or placed on finely perforated Teflon sheets or on sheets of silicon, e.g. with holes of an average diameter of 0.1- 1.5 cm, such as around 0.4-0.6 cm, and drying is then performed at a temperature of from about 20-40°C for a period of between 6 to 20 hours, in particular the temperature may be between 25°C to about 35°C, such as about 30°C. The period of time may in particular be for about 12 to about 24 hours, typically for about 16 hours. If desired, the resulting sponge material may be stabilised by treating it with dry heat at elevated temperatures, such as in the range of from about 110°C to 200°C. The stabilising time depends on the temperature, but will typically be from about 15 minutes to 6 hours. Stabilisation with dry heat may be carried out as described above.

[0114] In an alternative embodiment of the present disclosure, the sponge is prepared by freeze-drying a mixture of the two solutions.

[0115] In still another aspect the present disclosure relates to a method for preparing a haemostatic sponge comprising a biologically absorbable material and hyaluronic acid (HA) or a derivative thereof, wherein said HA, or a derivative thereof, is applied to one or more of the surfaces of the sponge, said method comprising the steps of:

i) providing a sponge comprising a biologically absorbable material;

ii) providing a solution of HA or a derivative thereof;

iii) applying said solution of HA, or a derivative thereof, to one or more of the surfaces of the sponge; and

iv) drying the resulting sponge.

[0116] Said method may in one embodiment of the present disclosure further comprise a step of stabilisation of the sponge, said stabilisation may in particular be carried out after one or more of the following steps: i), iii) and/or iv). The method of stabilisation may be any of those described below and includes but are not limited to treating it with dry and/or with a chemical cross-linking agent.

[0117] In particular the biologically absorbable material may be selected from the group consisting of gelatine, collagen, chitin, chitosan, alginate, cellulose, e.g. oxidised cellulose, regenerated oxidised cellulose, carboxymethylcellulose (CMC) or hydroxyethylcellulose (HEC), polyglycolic acid, polyacetic acid, derivatives thereof and mixtures thereof In a similar way as described above, the sponge is preferably a gelatine sponge, such as the commercially available Surgi-foam® sponge. The HA solution as well as the drying step is preferably as discussed above. The HA solution may be applied to one or more of the surfaces of the sponge by any conventional technique known to the person skilled in the art. Application of the HA or a derivative thereof may performed by methods such as spraying or painting the sponge.

[0118] The haemostatic powder or flakes according to the present disclosure may in one embodiment be prepared by milling a sponge of a biologically absorbable material and then mixing it with a powder or flakes of HA or a derivative thereof.

In another embodiment of the present disclosure it may be prepared by milling a sponge comprising a biologically absorbable material and hyaluronic acid or a derivative thereof. Milling of said sponges to a powder or flakes may be performed by methods known in the art, e.g. by means of rotary bed, extrusion, granulation and treatment in an intensive mixer, milling (e.g. by using a hammer mill or a centrifugal mill), or spray drying. Such powders or flakes may be used "as is" or may be pre-wetted with a liquid, such as saline, before use, thereby creating a paste. Preparation of such a paste may be performed as described above.

The sponges comprising a biologically absorbable material or a biologically absorbable material and HA or a derivative thereof may in particular have been stabilised.

[0119] The above-mentioned stabilisation step may unless stated otherwise be performed by any method. A preferred method of stabilisation of the sponges comprises heating the sponges at a temperature between 110-200°C. In particular the temperature may be in the range of between 110-160°C, e.g. in the range of between 110-140°C, or in the range of between 120-180°C, or in the range of between 130-170°C, or in the range of between 130-160°C, or in the range of between 120-150°C. The period of time depends on the temperature but may in particular be between 15 minutes and 6 hours, such as between 1-4 hours, e.g. between 1.5-3 hours, or between 2-4 hours.

However, other methods of stabilising such sponges include treating the sponges with a chemical cross-linking agent sometimes referred to as an activating agent.

Examples of such suitable cross-linking agents include but are not limited aldehydes, in particular glutaraldehyde and formaldehyde, acyl azide, caboiimides, hexamethylene diisocyanate, polyether oxide, 1,4-butanedioldiglycidyl ether, tannic acid, aldose sugars, e.g. D-fructose, genipin and dye-mediated photo-oxidation. Specific compounds include but are not limited to 1-(3-dimethylaminopropyl)-3-ethylcarboiimide hydrochloride (EDC), dithiobis(propanoic dihydrazide) (DTP), 1-ethyl-3-(3-dimethylamino-propyl)-carbodiimide (EDAC).

[0120] The step of drying referred to in the above-mentioned methods may in particular be performed at a temperature of from about 20-40°C, typically for a period of between 6 to 20 hours, in particular the temperature may be between 25°C to about 35°C, such as about 30°C. The period of time may in particular be for about 12 to about 24 hours, typically for about 16 hours.

[0121] The step of drying may in another embodiment of the present disclosure be carried out by freeze-drying.

[0122] All of the above-mentioned methods may comprise a further step of sterilisation, wherein said step generally takes place after the steps of the above-mentioned methods. Preferred methods of sterilisation include exposing the haemostatic composition to dry heat, ethylene oxide (EtO) or radiation, however other methods of sterilisation may be foreseen. Sterilisation with dry heat may typically be carried out by heating the sponges at a temperature between 110-200°C. In particular the temperature may be in the range of 110-160°C, e.g. in the range of 110-140°C, or in the range of 120-180°C, or in the range of 130-170°C, or in the range of 130-160°C, or in the range of 120-150°C. Furthermore, the period of time may in particular be for a period of between 15 minutes to 6 hours, such as between 1-4 hours, e.g. between 1,5-3 hours, or between 2-4 hours.

In another embodiment of the present disclosure sterilisation may be carried out by treating the sponge with EtO. Methods for sterilisation of such compositions with EtO are well known to a person skilled in the art.

In yet another embodiment of the present disclosure the sterilisation may be carried out by application of radiation, such as beta- or gamma-radiation. The dose typically lies in the range of 10-60 kGy, e.g. 20-60 kGy or 25-50 kGy, in particular

20 kGy or 25 kGy. Such treatment will reduce the bioburden of the sponge, and may also add to cross-linking of the molecular chains in the product. In one embodiment of the present disclosure the haemostatic composition is stabilised by treating it with dry heat. This treatment may also have the function of sterilisation so for this embodiment of the present disclosure the step of sterilisation may be the same as the step of stabilisation.
The present inventors have surprisingly discovered that sponges sterilised with dry heat or EtO appears to be more stable than sponges which have been sterilised with e-beam.

EXPERIMENTAL

[0123] In the following reference may be used interchangeably to the commercially available sponges Surgifoam® and Spongostan® which are the same.

Determination of water absorption according to USP 24

[0124] Cut a portion of about 10 mg from an absorbable gelatine sponge, weigh accurately, and place in a beaker of water. Knead gently between the fingers until thoroughly wet, and until all air has been removed, taking care not to break the tissue. Lift the portion of sponge from the water, and blot twice by pressing firmly between two pieces of absorbent paper. Drop the expressed sponge into a tared weighing bottle containing about 20 ml of water, and allow standing for 2 minutes. Lift the sponge from the water with a suitable hooked instrument, allow draining for 5 seconds, and discard the sponge. Again weigh the weighing bottle and water: the loss in water represents the weight of water absorbed by the sponge.

Porcine spleen model - Surgical procedure

[0125] The pig was the animal of choice because it has a large blood volume and a large vascular spleen that enables many haemostatic comparisons on each animal. The test compositions were applied one after one to surgical incisions made in the spleen. Negative controls were performed at the start and at the end of each study by using saline-moistened gauze to demonstrate consistent bleeding of >12 minutes in the absence of a haemostatic agent.
[0126] The primary test parameter was the time to haemostasis or reduction in the bleeding intensity.
[0127] A midline abdominal incision was made to expose the spleen. Incisions, 10-15 mm long and 2 mm deep, were made into the spleen. The test composition (or control) was applied with digital pressure for 2 minutes, the composition was lifted, and evaluation of the bleeding intensity was conducted. Subsequently, evaluation of the bleeding intensity was conducted every 30 seconds for 5-7 minutes.
[0128] About 20 incisions were made in each pig spleen.

Example 1 - Preparation of sponge #1

[0129] A HA gel (2% (w/v)) was prepared from *Streptococcus Equi* sp hyaluronic acid sodium salt (Biochemika) with a molecular weight of 1,500-1,800 kDa. The gel was added to freshly foamed gelatine (16.7% (w/v)). Immediately after addition of HA, the mixture was whirled at high velocity to avoid clogging of the gelatine. In order to avoid an inhomogeneous mixture, the temperature should not be below room temperature. After mixing, the mixture was poured into trays or placed on finely perforated teflon sheets, followed by air drying at approximately 30°C and 10% relative humidity for about 16 hours. Sponges prepared this way typically had a HA content of about 25-50% (w/w).

Example 2 - Preparation of sponge #2

[0130] A HA gel (2% (w/v)) was prepared from *Streptococcus Equi* sp hyaluronic acid sodium salt (Biochemika) with a molecular weight of 1,500-1,800 kDa. The gel was added to freshly foamed gelatine (16.7% (w/v)). Immediately after addition of HA, the mixture was whirled at high velocity to avoid clogging of the gelatine. In order to avoid an inhomogeneous mixture, the temperature should not be below room temperature. After mixing, the mixture was poured into trays or placed on fine perforated teflon sheets, followed by gentle freeze-drying (product temperature: 30°C after 7 hours). Sponges prepared this way had a more porous structure as compared to the sponges prepared in Example 1. Moreover, it was found that this method was suitable for preparing sponges having a higher thickness.

Example 3 - Preparation of sponge #3

[0131] HA gel (2 (w/v)) was prepared from *Streptococcus Equi* sp hyaluronic acid sodium salt (Biochemika) with a molecular weight of 1,500-1,800 kDa. The gel was smeared on the upper surface of a gelatine sponge (Surgifoam®)

followed by air drying at approximately 30°C and 10% relative humidity for about 16 hours. Sponges prepared this way typically had a HA content of at least 13% (w/w).

Example 4 - Determination of water absorption according to USP 24

[0132] Sponge #1 was subjected to the water absorption test (USP 24) described above. The USP 24 method is intended for absorbable gelatine sponges without HA. In the USP 24 method, kneading of the sponges has been optimised with respect to gelatine sponges. For the sponges as disclosed herein, the kneading should preferably be more gentle.

[0133] The absorption properties were compared to a commercially available gelatine sponge Surgifoam®. The following results were obtained:

	Sponge #1 Relative absorption (g/g)	Gelatine sponge Relative absorption (g/g)
	30.4	60.0
	29.5	57.9
	27.1	50.0
	24.1	54.9
	25.1	59.9
Mean:	27.2	56.5
SD:	2.7	4.0

[0134] As can be seen, the ratio between the water absorbed by sponge #1 and the water absorbed by the absorbable gelatine sponge (Surgifoam®) was 27.2/56.5 = 0.48.

[0135] In addition, by visual inspection of the wetted sponges, it was evident that sponge #1 swelled to a significantly lesser extent than did the Surgifoam® sponge.

Example 5 - Evaluation of haemostatic efficacy in a porcine spleen model

[0136] The object of this study was to compare the efficacy of the sponges of the present disclosure as compared to commercially available gelatine sponges (Surgifoam®) when applied to small, freely bleeding incisions made in the spleen of a pig. The sponges (6.5 cm^2) were applied after pre-wetting in sterile saline, followed by kneading as gently as possible.

[0137] The pig was the animal of choice since it has a large blood volume and a large vascular spleen that enables many haemostatic comparisons on each animal. The sponges were applied one after one to multiple surgical incisions in the spleen during the test period. The pigs were euthanized and not allowed to recover from anaesthesia.

[0138] The primary test parameter was time to haemostasis.

[0139] A midline abdominal incision was made to expose the spleen. 1.0 cm incisions (2 mm deep) were made in the spleen. Two incisions were made to demonstrate consistent bleeding with saline-moistened gauze and three incisions were made for each sponge to be tested.

[0140] The test sponge (or control) was applied with digital pressure for 2 minutes. Haemostasis evaluation occurred every 30 seconds with an additional 30 seconds of digital pressure. A negative control using saline-moistened gauze was performed at the start and at the end of the study to demonstrate consistent bleeding of >12 minutes in the absence of a haemostatic agent.

[0141] A trial was stopped if the bleeding did not stop within at least 5 minutes and/or if the sponge was saturated without reducing bleeding during the previous inspections.

[0142] The following results were obtained:

Sponge	Time to haemostasis (min)	Surgeon's comments
Negative control	>12	
Gelatine sponge	>6	Trial stopped because of saturated sponge and no reduced bleeding observed. The incision size was 1.5 cm in this trial. From this stage the incision size was reduced to 1.0 cm.

(continued)

Sponge	Time to haemostasis (min)	Surgeon's comments
Gelatine sponge	>5	Almost no bleeding. Trail stopped because of saturated sponge and no reduced bleeding was observed.
Gelatine sponge	>5	Trail stopped because of saturated sponge and no reduced bleeding was observed.
Sponge #1	5	Pre-wetting slow compared to Surgifoam®. Good adherence to surface. Only superior absorption in the sponge.
Sponge #1	3	Pre-wetting slow compared to Surgifoam® and becomes less soft by pre-wetting. Only superior absorption in the sponge.
Sponge #1	2	
Sponge #3*	>5.5	Reduced to moderate bleeding. Trial stopped because of saturated sponge and no reduced bleeding was observed.
Sponge #3*	>5	Trial stopped because of saturated sponge and no reduced bleeding was observed. Easy to pre-wet.
Sponge #3*	>5	Almost no bleeding. Product saturated after 5 minutes.
Sponge #3**	5	Only superficial absorption in the sponge.
Sponge #3**	5	Slight absorption within the sponge.
Sponge #3**	3	Only superficial absorption in the sponge
Negative control	>12	

*: HA layer away from bleeding
**: HA layer towards bleeding

[0143] The reason for Surgifoam® exhibiting a longer time to haemostasis than seen in previous porcine spleen models could be explained by the relative small size of the sponge (6.5 cm^2).

[0144] As can be seen from the obtained results, the haemostatic properties of the sponges of the present disclosure (sponge #1 and #3) were clearly superior as compared to Surgifoam®. In particular, sponge #1 was very efficient having a difference in mean time to haemostasis of at least 2 minutes as compared to Surgifoam®.

Example 6 - Evaluation of reduction of bleeding intensity in a porcine spleen model

[0145] The following different compositions were tested:

- A sponge of gelatine with and without HA (S4 vs. S1)
- A sponge of oxidised cellulose with and without HA (S9 vs. S8)
- A powder of gelatine with and without HA (S3 vs. S2)
- A sponge of gelatine with thrombin (S7)

[0146] The S1 sponge was a commercially available gelatine sponge Spongostan®.

[0147] The S4 sponge was prepared as described under example 1 and then subsequently treated with dry heat by placing it in a paper-bag in an oven at 150°C for 90 min.

[0148] The S7 sponge was a Spongostan® sponge, which was further soaked in a 1000 U/mL thrombin-solution.

[0149] The S8 sponge was a commercially available sponge of oxidised cellulose, called Surgicel®. The S9 sponge was prepared by wetting the commercially available sponge Surgicel® in a gel of HA until the concentration of HA in the sponge was 10% w/w. The sponge with HA was then subsequently freeze-dried.

[0150] The powder without HA (S2) was prepared by milling a Spongostan® sponge and just before application mixing the powder with saline to obtain a paste.

[0151] The powder with HA (S3) was prepared similarly by milling a Spongostan® sponge and then subsequently mixing the powder obtained by said milling with HA powder and then just before application adding saline to this mixture to obtain a paste.

[0152] In short, the haemostatic efficacy of the samples was evaluated by the following method: An incision was made into the spleen and the intensity of the resulting bleeding was evaluated on a scale ranging from 0-5. Subsequently, the sample in question was applied to the incision and at predetermined intervals, the sample was lifted from the incision and the intensity of the bleeding was evaluated before the sample was reapplied. Powder samples and oxidised cellulose samples was not lifted from the incision which is very likely to affect the reduction in the bleeding intensity also for the compositions without HA. The bleeding intensity was in those cases evaluated by the amount of blood leaking through the samples. Each experiment was terminated after 7 minutes if haemostasis (0 on the scale) had not occurred before. 7 repetitions (distributed onto all four pigs) were performed for each type of sample, except for the reference samples for which 3 repetitions were performed on each pig, i.e. 12 repetitions in total.

[0153] The bleeding intensity reduction was calculated for each experiment as the difference between the evaluated bleeding intensities at the start and at the end of the experiment. The resulting averages of these numbers are shown in below Table.

Results:

Sample	Average bleeding intensity reduction	Lifting of the composition from the incision for evaluation of the bleeding intensity
S1: Gelatine sponge (Spongostan®), heated at 150°C for 180 min	0,82	Yes
S4: Gelatine sponge with 30% w/w HA (Biochemika), heated at 150°C for 90 min	4,29	Yes
S7: Gelatine sponge (Spongostan®) with thrombin	3,00	Yes
S2: Gelatine powder irradiated (e-beam) with 15 kGy	2,86	No
S3: Gelatine powder with 10% w/w HA (HTL) and irradiated (e-beam) with 15 kGy	3,67	No
S8: Sponge of oxidised cellulose (Surgicel®)	2,71	No
S9: Sponge of oxidised cellulose (Surgicel®) with 10% w/w HA (HTL)	3,29	No

[0154] The results show that the presence of a gelatine sponge, a gelatine powder and a sponge of oxidised cellulose with HA reduce the bleeding intensity more than the same sponges and powder without HA (S4 vs. S1, S3 vs. S2 and S9 vs. S8).
Furthermore, the results also show that a gelatine sponge with HA results in a larger reduction in the bleeding intensity than a gelatine sponge with thrombin (S4 vs. S7).

[0155] In a similar study the reduction of the bleeding intensity of a Lyostypt collagen compress (commercially available) without HA or with 10% w/w HA was measured. For the Lyostypt collagen compress without HA the average reduction in the bleeding intensity was found to be 1.00, while for the Lyostypt collagen compress with 10% w/w HA it was found to be 1.43. Thus also for this type of haemostatic composition the presence of HA appears to improve the reduction in the bleeding intensity.
The Lyostypt collagen compress was not lifted from the incision for evaluation of the bleeding intensity.

Example 7 - Evaluation of the time to haemostasis in a porcine spleen model; effect of method of application and the presence of thrombin in the sponge

[0156] The sponges were prepared as described in example 1. Subsequently each of the sponges was treated in the following manner:

Test sample B was moistened and kneaded in a jar with plenty of sterile saline for 30 seconds and squeezed before

application. Test sample B was before application placed on pre-wetted gauze.
Test samples D and E were placed in a jar with 2 mL sterile saline. The sponge was kneaded very gently and turned upside down to make sure all the saline was absorbed within the whole sponge. After a maximum of 1 minute the bottom-side of the sponge was placed upside on the Parafilm topfilm and was ready for application.
Test samples F and G were placed in a jar with 2 mL Thrombin solution (1000 U/mL) and the sponges were kneaded very gentle and turned upside down to make sure all the Thrombin solution was absorbed within the whole sponge. After a maximum of 1 minute the bottom-side of the sponge was placed upside on the Parafilm topfilm and then it was ready for application.
Test samples J, and K) were applied dry.
Test sample L was wetted in saline as described for test sample D and E.

Results:

Sponge	Time to haemostasis
B: Gelatine sponge (Surgifoam®) wetted in saline	3-5 minutes
D: Gelatine sponge with 30% w/w HA with a Mw of 2,18 x 10^5 Da wetted in saline	2,5-3,5 min (average 3 min)
E: Gelatine sponge with 30% w/w HA with a Mw of 1,06 x 10^6 Da wetted in saline	2,0-2,5 min (average 2,25 min)
F: Gelatine sponge ith 30% w/w HA with a Mw of 2,18 x 10^5 Da wetted in 1000 units/ml thrombin	2,0-3,0 (average 2,25 min)
G: Gelatine sponge with 30% w/w HA with a Mw of 1,06 x 10^6 Da wetted in 1000 units/ml thrombin	2,0 min (average 2,0 min)
J: Gelatine sponge with 30% w/w HA with a Mw of 2,18 x 10^5 Da applied dry	4,0-7,0 min (average 5,75 min)
K: Gelatine sponge with 30% w/w HA with a Mw of 1,06 x 10^6 Da applied dry	5 min to 7 min
L: Gelatine sponge with 30% w/w HA from a fermented process and with a Mw of 1,5-1,8 x 10^6 Da wetted in saline	2,0-2,5 min (average 2,25 min)

[0157] The results show that when the sponges are applied wet the time to haemostasis appears to be shorter than when the sponges are applied dry (D vs. J and E vs. K).
Furthermore, wetting the sponges in thrombin appears to reduce the time to haemostasis even further than by wetting them in saline (F vs. D and G vs. E).
The higher molecular weight of HA also appears to reduce the time to haemostasis more than the lower molecular weight of HA (E vs. D and G vs. F).

Example 8: Use of a sponge of the present disclosure as a vehicle for delivery of a substance

[0158] The sponge of the present disclosure was compared to the commercially available Surgifoam® with respect of release of tobramycin sulphate.
[0159] Both preparations were cut into smaller sponges with a diameter of 14 mm and soaked in a solution of tobramycin sulphate. The release of tobramycin sulphate from the preparations serving as vehicles, were measured by quantification of tobramycin sulphate using an USP HPLC method. The actual release took place in Franz diffusion cells, and samples were taken after 0, 10, 30, 60, 120, 1440, 2880 and 3975 minutes. The amount of tobramycin sulphate was calculated on the basis of a standard solution.
[0160] The results were that the sponges comprising gelatin and HA had a tendency to release more tobramycin sulphate that the commercially available gelatin sponge did in the 3975 minutes the study ran.
[0161] Additionally a collagen sponge comprising 10% HA w/w was investigated in the same set up. As for the above, the results indicated that the release of tobramycin sulphate was greater from the collagen sponge with HA than from the gelatin sponge (Surgifoam®)
[0162] Furthermore the release of carboplatin from the present investigation, the colla-gen sponge with 10% HA w/w and the commercially available Surgifoam® was examined in a similar study. The results obtained with release of carboplatin con-firmed the tendency seen with tobramcyin that the preparations containing HA releases more of the test substance than the gelatin sponge does.
[0163] The results of the above indicate that the sponge of the present disclosure and a collagen sponge comprising HA can be used as vehicles for different substances, and that their release seems to be greater than from a gelatin sponge.

Claims

1. A haemostatic composition comprising gelatine and hyaluronic acid or a derivative thereof,
wherein said hyaluronic acid or derivative thereof, is incorporated into said composition to a final content of at least 10% (w/w) hyaluronic acid,
wherein said composition does not comprise a chemical cross-linking agent or residues thereof, and
wherein said composition is obtained by treating a mixture of gelatine, hyaluronic acid or a derivative thereof and a solvent with dry heat at a temperature of from 110 to 200°C.

2. The haemostatic composition according to claim 1, wherein the haemostatic composition is in the form of a sponge, a powder or flakes.

3. The haemostatic composition according to claim 2, wherein the haemostatic composition is a sponge.

4. The haemostatic composition according to any of claims 1 -3, wherein said composition comprises at least 15% (w/w) of hyaluronic acid or a derivative thereof, such as at least 20% (w/w) of hyaluronic acid or a derivative thereof, e.g. at least 25% (w/w) of hyaluronic acid or a derivative thereof, preferably at least 30% (w/w) of hyaluronic acid or a derivative thereof, such as at least 35% (w/w) of hyaluronic acid or a derivative thereof, e.g. at least 40% (w/w) of hyaluronic acid or a derivative thereof.

5. The haemostatic composition according to any of claims 1 to 4, wherein said hyaluronic acid derivative is a salt or an ester of hyaluronic acid.

6. The haemostatic composition according to any of the previous claims, wherein said composition comprises at the most 90% (w/w) of said gelatine, or at the most 85% (w/w) of said gelatine, such as at the most 80% (w/w) of said gelatine, e.g. at the most 75% (w/w) of said gelatine, preferably at the most 70% (w/w) of said gelatine, such as at the most 65% (w/w) of said gelatine, e.g. at the most 60% (w/w) of said gelatine.

7. The haemostatic composition according to any of the previous claims, which further comprises at least one blood coagulation factor, wherein said blood coagulation factor is selected from the group consisting of thrombin or a precursor thereof, factor Va, factor VIIa, factor VIIIa, factor IXa, factor Xa, factor XIa, factor XIIa, factor XIIIa and calcium ions.

8. The haemostatic composition according to claim 7, which further comprises a thrombin-stabilising agent selected from the group consisting of naturally occurring amino acids, mono-, di- or polysaccharides, polyglycols, proteins and mixtures thereof.

9. The haemostatic composition according to any of the previous claims, which further comprises at least one anti-fibrinolytic agent, wherein said anti-fibrinolytic agent is selected from the group consisting of aprotinin, pepstatin, leupeptin, antipain, chymostatin, gabexate mesilate, fibronectin, ε-amino caproic acid and tranexamic acid.

10. The haemostatic composition according to claim 3, wherein the sponge absorbs less water than an absorbable gelatine sponge.

11. The haemostatic composition according to claim 3, wherein at least one surface of the sponge is covered by a top sheet.

12. The haemostatic composition according to any of the previous claims, wherein the hyaluronic acid or a derivative thereof has a molecular weight in the range of from 50 to 5,000 kDa, such as in the range of from 50 to 4,000 kDa, for example in the range from 100 to 3,000 kDa, such as in the range of from 250 to 3,500 kDa, for example in the range from 500 to 2,500 kDa, such as in the range of from 500 to 2,000 kDa.

13. The haemostatic composition according to any of the previous claims, wherein the hyaluronic acid has a pH value in the range of from 5 to 9, such as from 6 to 8, for example 6.5 to 7.5, such as about 7.

14. The haemostatic composition according to claim 3, which further comprises one or more antimicrobial agent(s).

15. The haemostatic composition according to claim 3, which further comprises one or more desirable agent(s), wherein

said one or more desirable agent(s) is selected from the group consisting of surfactants, antiseptics, pain relieving agents, chemotherapeutics, anaesthetics, healing-promoting agents, vitamins, minerals, amino acids, proteins, growth factors, cells, enzymes, contrast agents, preservatives, emulsifiers and chemotherapeutic agents.

16. The haemostatic composition according to any of the previous claims for use as a haemostatic adjunct in medical, veterinary or dental surgery.

17. The haemostatic composition according to any of claims 1 to 15 for use as a vehicle for the delivery of an agent.

18. A method of producing a haemostatic composition comprising the steps of:

i) mixing hyaluronic acid or a derivative thereof, a solvent and gelatine;
ii) treating the mixture obtained in step i) with dry heat at a temperature between 110-200°C.

19. The method of claim 18, wherein said dry heat treatment at a temperature of from 110 to 200°C is conducted for 15 minutes to 6 hours.

20. The method of any of claims 18 to 19, wherein said method comprises a further step of drying the mixture obtained in step i) before the mixture is treated in step ii).

21. The method of any of claims 18 to 20, wherein the mixing of step i) is performed by any of the following alternatives:

a) mixing gelatine with hyaluronic acid or a derivative thereof and then subsequently adding a solvent;
b) mixing a solution of gelatine with a solution of hyaluronic acid or a derivative thereof;
c) mixing gelatine with a solution of hyaluronic acid or a derivative thereof;
d) mixing a solution of gelatine with hyaluronic acid or a derivative thereof.

22. The method of any of claims 18 to 21, wherein said mixing is performed under mechanical influence, such as whipping, stirring, spinning, static mixing, motionless mixing or centrifugation.

23. The method of any of claims 18 to 22, wherein said method further comprises a step of sterilization of the composition.

24. The method of any of claims 19 to 23, wherein said drying is performed at a temperature from about 20°C to about 40°C for a period of between 6 to 20 hours.

25. The method of any of claims 19 to 23, wherein said drying is performed by freeze-drying.

26. A haemostatic paste prepared by wetting the powder or flakes according to claim 2 with a liquid, thereby creating the paste.

Patentansprüche

1. Hämostatische Zusammensetzung, die Gelatine und Hyaluronsäure oder ein Derivat davon umfasst,
wobei die Hyaluronsäure oder das Derivat davon zu einem Endgehalt von mindestens 10 % (Gew./Gew.) Hyaluronsäure in die Zusammensetzung inkorporiert ist,
wobei die Zusammensetzung kein chemisches Vernetzungsmittel oder Rückstände davon umfasst, und
wobei die Zusammensetzung durch Behandeln eines Gemischs aus Gelatine, Hyaluronsäure oder einem Derivat davon und einem Lösungsmittel mit trockener Hitze bei einer Temperatur von 110 bis 200°C erhalten wird.

2. Hämostatische Zusammensetzung nach Anspruch 1, wobei die hämostatische Zusammensetzung in Form eines Schwammes, eines Pulvers oder von Flocken ist.

3. Hämostatische Zusammensetzung nach Anspruch 2, wobei die hämostatische Zusammensetzung ein Schwamm ist.

4. Hämostatische Zusammensetzung nach einem der Ansprüche 1 -3, wobei die Zusammensetzung mindestens 15 % (Gew./Gew.) Hyaluronsäure oder eines Derivats davon umfasst, wie etwa mindestens 20 % (Gew./Gew.) Hyaluronsäure oder eines Derivats davon, z. B. mindestens 25 % (Gew./Gew.) Hyaluronsäure oder eines Derivats davon,

vorzugsweise mindestens 30 % (Gew./Gew.) Hyaluronsäure oder eines Derivats davon, wie etwa mindestens 35 % (Gew./Gew.) Hyaluronsäure oder eines Derivats davon, z. B. mindestens 40 % (Gew./Gew.) Hyaluronsäure oder eines Derivats davon.

5. Hämostatische Zusammensetzung nach einem der Ansprüche 1 bis 4, wobei das Hyaluronsäure-Derivat ein Salz oder ein Ester der Hyaluronsäure ist.

6. Hämostatische Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei die Zusammensetzung höchstens 90 % (Gew./Gew.) Gelatine umfasst oder höchstens 85 % (Gew./Gew.) Gelatine, wie etwa höchstens 80 % (Gew./Gew.) Gelatine, z. B. höchstens 75 % (Gew./Gew.) Gelatine, vorzugsweise höchstens 70 % (Gew./Gew.) Gelatine, wie etwa höchstens 65 % (Gew./Gew.) Gelatine, z. B. höchstens 60 % (Gew./Gew.) Gelatine.

7. Hämostatische Zusammensetzung nach einem der vorhergehenden Ansprüche, die weiter mindestens einen Blutgerinnungsfaktor umfasst, wobei der Blutgerinnungsfaktor aus der Gruppe bestehend aus Thrombin oder einem Vorläufer davon, Faktor Va, Faktor VIIa, Faktor VIIIa, Faktor IXa, Faktor Xa, Faktor XIa, Faktor XIIa, Faktor XIIIa und Calciumionen ausgewählt ist.

8. Hämostatische Zusammensetzung nach Anspruch 7, die weiter ein Thrombin-Stabilisierungsmittel umfasst, dass aus der Gruppe bestehend aus natürlich vorkommenden Aminosäuren, Mono-, Di- oder Polysacchariden, Polyglykolen, Proteinen und Gemischen davon ausgewählt ist.

9. Hämostatische Zusammensetzung nach einem der vorhergehenden Ansprüche, die weiter mindestens ein antifibrinolytisches Mittel umfasst, wobei das antifibrinolytische Mittel aus der Gruppe bestehend aus Aprotinin, Pepstatin, Leupeptin, Antipain, Chymostatin, Gabexatmesilat, Fibronektin, ε-Aminocapronsäure und Tranexamsäure ausgewählt ist.

10. Hämostatische Zusammensetzung nach Anspruch 3, wobei der Schwamm weniger Wasser als ein absorbierbarer Gelatineschwamm absorbiert.

11. Hämostatische Zusammensetzung nach Anspruch 3, wobei mindestens eine Oberfläche des Schwamms von einer oberen Folie abgedeckt ist.

12. Hämostatische Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei die Hyaluronsäure oder ein Derivat davon ein Molekulargewicht im Bereich von 50 bis 5.000 kDa aufweist, wie etwa im Bereich von 50 bis 4.000 kDa, beispielsweise im Bereich von 100 bis 3.000 kDa, wie etwa im Bereich von 250 bis 3.500 kDa, beispielsweise im Bereich von 500 bis 2.500 kDa, wie etwa im Bereich von 500 bis 2.000 kDa.

13. Hämostatische Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei die Hyaluronsäure einen pH-Wert im Bereich von 5 bis 9 aufweist, wie etwa von 6 bis 8, beispielsweise 6, 5 bis 7,5, wie etwa 7.

14. Hämostatische Zusammensetzung nach Anspruch 3, die weiter ein oder mehrere antimikrobielle(s) Mittel umfasst.

15. Hämostatische Zusammensetzung nach Anspruch 3, die weiter ein oder mehrere wünschenswerte(s) Mittel umfasst, wobei das eine oder die mehreren wünschenswerte(n) Mittel aus der Gruppe bestehend aus Tensiden, Antiseptika, schmerzlindernden Mitteln, Chemotherapeutika, Anästhetika, heilungsfördernden Mitteln, Vitaminen, Mineralstoffen, Aminosäuren, Proteinen, Wachstumsfaktoren, Zellen, Enzymen, Kontrastmitteln, Konservierungsmitteln, Emulgatoren und chemotherapeutischen Mitteln ausgewählt ist bzw. sind.

16. Hämostatische Zusammensetzung nach einem der vorhergehenden Ansprüche zur Verwendung als hämostatisches Zusatzmittel bei medizinischen, tiermedizinischen oder zahnmedizinischen Eingriffen.

17. Hämostatische Zusammensetzung nach einem der Ansprüche 1 bis 15 zur Verwendung als Vehikel für die Abgabe eines Mittels.

18. Verfahren zur Herstellung einer hämostatischen Zusammensetzung, die folgenden Schritte umfassend:

i) Vermischen von Hyaluronsäure oder eines Derivats davon, eines Lösungsmittels und Gelatine;
ii) Behandeln des in Schritt i) erhaltenen Gemischs mit trockener Hitze bei einer Temperatur zwischen

110-200°C.

19. Verfahren nach Anspruch 18, wobei die Trockenwärmebehandlung bei einer Temperatur von 110-200°C 15 Minuten bis 6 Stunden lang durchgeführt wird.

20. Verfahren nach einem der Ansprüche 18 bis 19, wobei das Verfahren einen weiteren Schritt des Trocknens des in Schritt i) erhaltenen Gemischs umfasst, bevor das Gemisch in Schritt ii) behandelt wird.

21. Verfahren nach einem der Ansprüche 18 bis 20, wobei das Vermischen von Schritt i) durch eine der folgenden Alternativen durchgeführt wird:

a) Vermischen von Gelatine mit Hyaluronsäure oder einem Derivat davon und dann anschließend Zugeben eines Lösungsmittels;
b) Vermischen einer Lösung von Gelatine mit einer Lösung von Hyaluronsäure oder einem Derivat davon;
c) Vermischen von Gelatine mit einer Lösung von Hyaluronsäure oder einem Derivat davon;
d) Vermischen einer Lösung von Gelatine mit Hyaluronsäure oder einem Derivat davon.

22. Verfahren nach einem der Ansprüche 18 bis 21, wobei das Vermischen unter mechanischer Einwirkung durchgeführt wird, wie etwa Schlagen, Rühren, Drehen, statisches Vermischen, bewegungsarmes Vermischen oder Zentrifugieren.

23. Verfahren nach einem der Ansprüche 18 bis 22, wobei das Verfahren weiter einen Schritt der Sterilisierung der Zusammensetzung umfasst.

24. Verfahren nach einem der Ansprüche 19 bis 23, wobei das Trocknen bei einer Temperatur von etwa 20°C bis etwa 40°C für einen Zeitraum zwischen 6 bis 20 Stunden durchgeführt wird.

25. Verfahren nach einem der Ansprüche 19 bis 23, wobei das Trocknen durch Gefriertrocknen durchgeführt wird.

26. Hämostatische Paste, hergestellt durch Benetzen des Pulvers oder der Flocken nach Anspruch 2 mit einer Flüssigkeit, um dadurch die Paste zu schaffen.

Revendications

1. Composition hémostatique comprenant de la gélatine et de l'acide hyaluronique ou l'un de ses dérivés, dans laquelle ledit acide hyaluronique ou son dérivé, est incorporé dans ladite composition jusqu'à une teneur finale d'au moins 10 % (p/p) d'acide hyaluronique,
dans laquelle ladite composition ne comprend pas d'agent de réticulation chimique ni de résidus de celui-ci, et dans laquelle ladite composition est obtenue par traitement d'un mélange de gélatine, d'acide hyaluronique ou de l'un de ses dérivés et d'un solvant avec une chaleur sèche à une température de 110 à 200 °C.

2. Composition hémostatique selon la revendication 1, dans laquelle la composition hémostatique est sous la forme d'une éponge, d'une poudre ou de flocons.

3. Composition hémostatique selon la revendication 2, dans laquelle la composition hémostatique est une éponge.

4. Composition hémostatique selon l'une quelconque des revendications 1 à 3, dans laquelle ladite composition comprend au moins 15 % (p/p) d'acide hyaluronique ou de l'un de ses dérivés, comme au moins 20 % (p/p) d'acide hyaluronique ou de l'un de ses dérivés, par exemple, au moins 25 % (p/p) d'acide hyaluronique ou de l'un de ses dérivés, de préférence au moins 30 % (p/p) d'acide hyaluronique ou de l'un de ses dérivés, comme au moins 35 % (p/p) d'acide hyaluronique ou de l'un de ses dérivés, par exemple, au moins 40 % (p/p) d'acide hyaluronique ou de l'un de ses dérivés.

5. Composition hémostatique selon l'une quelconque des revendications 1 à 4, dans laquelle ledit dérivé d'acide hyaluronique est un sel ou un ester d'acide hyaluronique.

6. Composition hémostatique selon l'une quelconque des revendications précédentes, dans laquelle ladite composition

comprend au plus 90 % (p/p) de ladite gélatine, ou au plus 85 % (p/p) de ladite gélatine, comme au plus 80 % (p/p) de ladite gélatine, par exemple, au plus 75 % (p/p) de ladite gélatine, de préférence au plus 70 % (p/p) de ladite gélatine, comme au plus 65 % (p/p) de ladite gélatine, par exemple, au plus 60 % (p/p) de ladite gélatine.

7. Composition hémostatique selon l'une quelconque des revendications précédentes, qui comprend en outre au moins un facteur de la coagulation sanguine, dans laquelle ledit facteur de la coagulation sanguine est choisi dans le groupe constitué de la thrombine ou l'un de ses précurseurs, le facteur Va, le facteur VIIa, le facteur VIIIa, le facteur IXa, le facteur Xa, le facteur XIa, le facteur XIIa, le facteur XIIIa et les ions calcium.

8. Composition hémostatique selon la revendication 7, qui comprend en outre un agent stabilisant la thrombine choisi dans le groupe constitué d'acides aminés existant à l'état naturel, de mono-, di- ou polysaccharides, de polyglycols, de protéines et de leurs mélanges.

9. Composition hémostatique selon l'une quelconque des revendications précédentes, qui comprend en outre au moins un agent antifibrinolytique, dans laquelle ledit agent antifibrinolytique est choisi dans le groupe constitué de l'aprotinine, la pepstatine, la leupeptine, l'antipaïne, la chymostatine, le mésylate de gabexate, la fibronectine, l'acide ε-aminocaproïque et l'acide tranexamique.

10. Composition hémostatique selon la revendication 3, dans laquelle l'éponge absorbe moins d'eau qu'une éponge de gélatine absorbable.

11. Composition hémostatique selon la revendication 3, dans laquelle au moins une surface de l'éponge est couverte d'un feuillet supérieur.

12. Composition hémostatique selon l'une quelconque des revendications précédentes, dans laquelle l'acide hyaluronique ou l'un de ses dérivés a un poids moléculaire situé dans la plage de 50 à 5 000 kDa, comme dans la plage de 50 à 4 000 kDa, par exemple dans la plage de 100 à 3 000 kDa, comme dans la plage de 250 à 3 500 kDa, par exemple dans la plage de 500 à 2 500 kDa, comme dans la plage de 500 à 2 000 kDa.

13. Composition hémostatique selon l'une quelconque des revendications précédentes, dans laquelle l'acide hyaluronique a une valeur de pH de 5 à 9, comme de 6 à 8, par exemple, 6,5 à 7,5, comme environ 7.

14. Composition hémostatique selon la revendication 3, qui comprend en outre un ou plusieurs agents antimicrobiens.

15. Composition hémostatique selon la revendication 3, qui comprend en outre un ou plusieurs agents souhaitables, dans laquelle lesdits un ou plusieurs agents souhaitables sont choisis dans le groupe constitué de tensioactifs, antiseptiques, agents soulageant la douleur, agents chimiothérapeutiques, anesthésiques, agents favorisant la cicatrisation, vitamines, minéraux, acides aminés, protéines, facteurs de croissance, cellules, enzymes, agents de contraste, conservateurs, émulsifiants et agents chimiothérapeutiques.

16. Composition hémostatique selon l'une quelconque des revendications précédentes pour une utilisation en tant que complément hémostatique en chirurgie médicale, vétérinaire ou dentaire.

17. Composition hémostatique selon l'une quelconque des revendications 1 à 15, pour une utilisation en tant que véhicule pour l'administration d'un agent.

18. Procédé de production d'une composition hémostatique comprenant les étapes suivantes :

i) le mélange d'acide hyaluronique ou de l'un de ses dérivés, d'un solvant et de gélatine ;
ii) le traitement du mélange obtenu dans l'étape i) avec une chaleur sèche à une température située entre 110 et 200 °C.

19. Procédé selon la revendication 18, dans lequel ledit traitement à la chaleur sèche à une température de 110 à 200 °C est conduit pendant 15 minutes à 6 heures.

20. Procédé selon l'une quelconque des revendications 18 à 19, dans lequel ledit procédé comprend une autre étape de séchage du mélange obtenu dans l'étape i) avant le traitement du mélange dans l'étape ii).

21. Procédé selon l'une quelconque des revendications 18 à 20, dans lequel le mélange de l'étape i) est effectué par l'une quelconque des variantes suivantes :

a) le mélange de la gélatine avec l'acide hyaluronique ou l'un de ses dérivés et ensuite l'ajout subséquent d'un solvant ;
b) le mélange d'une solution de gélatine avec une solution d'acide hyaluronique ou de l'un de ses dérivés ;
c) le mélange de la gélatine avec une solution d'acide hyaluronique ou de l'un de ses dérivés ;
d) le mélange d'une solution de gélatine avec de l'acide hyaluronique ou l'un de ses dérivés.

22. Procédé selon l'une quelconque des revendications 18 à 21, dans lequel ledit mélange est effectué sous influence mécanique, comme un fouettage, une agitation, une rotation, un mélange statique, un mélange sans mouvement ou une centrifugation.

23. Procédé selon l'une quelconque des revendications 18 à 22, dans lequel ledit procédé comprend en outre une étape de stérilisation de la composition.

24. Procédé selon l'une quelconque des revendications 19 à 23, dans lequel ledit séchage est effectué à une température d'environ 20 °C à environ 40 °C pendant une période située entre 6 et 20 heures.

25. Procédé selon l'une quelconque des revendications 19 à 23, dans lequel ledit séchage est effectué par cryodessiccation.

26. Pâte hémostatique préparée par mouillage de la poudre ou des flocons selon la revendication 2 avec un liquide, créant de cette façon la pâte.

REFERENCES CITED IN THE DESCRIPTION

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